another by a suitable number of the detector pixels to avoid image overlap. For instance, in typical implementations for imaging cells having nominally 10 micron diameters, the 2T1T object field of view 164 is configured to be approximately 90 microns. Given an exemplary 10 pixel separation between the 2T1T focus area 230 and the 2R defocused focus area 234 and with an exemplary implementation of the first detector 120 having 13 micron sized pixels, a satisfactory channel separation would be 100 pixels or 1.3 mm. Furthermore, as an example, if the imaging system 100 were to have an overall magnification of 40X, and focal length of 200 mm for the imaging lens 118, the optical angle of separation 142 between the imaged 2T1T light 144 and the imaged 2R defocused light 150 would be approximately 6.5 milliradians. Consequently, in this example, the mechanical angle 104 for the amplitude beam splitter 114 would be approximately 3.25 milliradians.

Another implementation of the imaging system 100, illustrated in Figures 17 and 18, uses a spectral dispersing element 246, such as a prism or diffraction grating, to spectrally disperse light from the amplitude beam splitter 114, shown in Figure 17, or from the polarization beam splitter 208, not shown in Figure 17, such as the 2T1T light 138 and the 2R defocused light to transmit spectrally dispersed 2T1T light 248 and spectrally dispersed 2R defocused light 250. The imaging lens 118 then receives the spectrally dispersed 2T1T light 248 and spectrally dispersed 2R defocused light 250 to transmit imaged, spectrally dispersed 2T1T light 252 and imaged, spectrally dispersed 2R defocused light 254, respectively, having the 2T1T image plane 146 and the 2R defocused image plane 152, with respect to a common point on target object 102, respectively. As illustrated in Figure 19, the 2T1T focus area 230 and the 2R defocused focus area 234 have a spectrally dispersed band of images, 2T1T focus cell dispersed image set 256 and 2R defocused focus cell dispersed image set 258, respectively, for each occurrence of the target object 102. This spectral dispersion is useful for analysis of the target object 102.

Another implementation of the imaging system 100, illustrated in Figure 20, uses an x-axis imaging system 260 and an y-axis imaging system 262 to image the target object 102 bi-dimensionally from two different orientations, which is useful, for instance,

to distinguish features that may otherwise overlap when viewed from a single orientation. The particular implementation illustrated in Figure 20 utilizes polarization effects in conjunction with the optical retardation plate 212 and the polarization beam splitter 208 and spectral dispersion effects in conjunction with the spectral dispersing element 246. However, other implementations can use the x-axis imaging system 260 and the y-axis imaging system 262 with or without the polarization effects and the spectral dispersion effects.

Applications of bi-dimensional implementations of the imaging system 100 include analyzing multi-component objects in solution, such as cells containing FISH probes. Since FISH probes appear as point sources of light within the three-dimensional nucleus of a cell, in some cases, two or more FISH probes may appear in an overlapping relationship along the optical axis of the imaging system. Consequently, one or more FISH probes may obscure one or more other FISH probes to undermine attempts at determining the quantity of FISH probes contained within a cell. Determination of FISH probe quantity within a cell has tremendous utility such as in determining genetic abnormalities, (for example, trisomy 21, otherwise known as Down's syndrome).

By positioning the optical axes of the x-axis imaging system 260 and the y-axis imaging system 262 so that they are oriented with respect to one another by 90°, such as the optical axis of the x-axis imaging system being along the x-axis and the optical axis of the y-axis imaging system being along the y-axis, as shown in Figure 20, it is possible to separately resolve image spots imaged from corresponding two or more FISH probe objects on at least one of the first detectors 120 of at least one of the x-axis imaging system and the y-axis imaging system. It has been found that if two or more FISH probes overlap in regard to the image produced on one of the first detectors 120, the two or more FISH probes can be separately resolved in the spectrally dispersed images produced on the other first detector.

This is in contrast to conventional approaches where single-orientation systems may address problems caused by image overlap due to defocus by panning through objects along the optical axis of the conventional systems to acquire multiple image planes

in the object. These conventional approaches require significant amounts of time to collect multiple images and cannot readily be applied to objects, such as cells, in flow. The implementation of the imaging system 100 using two imaging sub-systems, the x-axis imaging system 260 and the y-axis imaging system 262, addresses image overlap problems, even while objects to be imaged are in motion, through its multi-object plane approach.

Object planes associated with an orthogonal orientation of the optical axis of the x-axis imaging system 260 with respect to the y-axis imaging system 262 are illustrated in Figure 22. As a result of the orthogonal orientation of the optical axis of the x-axis imaging system 260 with respect to the y-axis imaging system 262, the 2T1T object plane 156 and the 2R defocused object plane 158 of the x-axis imaging system are also orthogonal with respect to the 2T1T object plane and the 2R defocused object plane of the y-axis imaging system.

In an alternative implementation of the imaging system 100 as a bi-oriented imaging system 264, illustrated in Figure 23, a focus feedback error is generated to dynamically acquire or maintain focus. The bi-oriented imaging system 264 includes a flow cell cuvette 266, a flow cell cavity 268, an illumination light 270, a first imaging sub-system 272, a first detector 274, second imaging sub-system 276, a second detector 278 and a processor 280. The first imaging sub-system 272 receives the first collected light from a second target object 282 and transmits first focused light 284 along a first optical axis 286 to be received by the first detector 274. The first focused light 284 has a first imaging sub-system best focused conjugate image for second target object (first image of second target) 288 with respect to the second target object 282. The first focused light 284 also has a first imaging sub-system best focused conjugate image for first target object (first image of first target) 290 with respect to a first target object 292 also in the flow cell cavity 268. The first collected light results from light either being emanated from luminous versions of the second target object 282 or coming from an incoherent or coherent light source and being scattered or reflected off of the second target object. The second imaging sub-system 276 receives the second collected light from the second target object 282 and transmits second focused light 294 along second optical axis 296 to be focused at the